RESEARCH

Multiple natural enemies do not improve two spotted spider mite and flower western thrips control in strawberry tunnels

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Biological control techniques are commonly used in many horticultural crops in Spain, however the application of these techniques to Spanish strawberries are relatively recent. In this study the effectiveness of augmentative biological control techniques to control the two main strawberry (*Fragaria ×ananassa* Duchesne) pest: the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychidae), and the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), through releases of the predatory mites *Phytoseiulus persimilis* Athias-Henriot, *Neoseiulus californicus* (McGregor), *Amblyseius swirskii* Athias-Henriot (Acari: Phytoseiidae), and *Orius laevigatus* (Fieber) (Heteroptera: Anthocoridae) were tested. Two-year results on the performance of treatments using combinations of these biocontrol agents are presented. In both years, all treatments resulted in the reduction of TSSM numbers; but no treatment was better than the release of *P. persimilis* alone ($P < 0.05$). TSSM suppression varied among crop phases being greater early in the season. None of the treatments reduced significantly WFT numbers ($P < 0.05$), and the established economic injury level (EIL) was surpassed from March to late April in both years. However, EIL was surpassed less times when treatment included *O. laevigatus* (2009: 20.7%; 2010: 22.7% of samples). No effect of *A. swirskii* was observed when this mite was released. Results corroborate that biological control techniques for TSSM and WFT are feasible for high-plastic tunnel strawberries. Under the conditions in our study no additive effects were observed, and there was not advantage in the release of multiple natural enemies.

Key words: Biological control, combined releases, *Fragaria×ananassa*, *Frankliniella occidentalis*, *Orius laevigatus*, Phytoseiids, *Tetranychus urticae*.

INTRODUCTION

The strawberry (*Fragaria ×ananassa* Duchesne, Rosaceae) is one of the most consumed berry fruit worldwide having experienced one of the highest growth rates of consumption of all fruit and vegetables over the two last decades. The USA is the world's largest producer of strawberries, producing over 1.4 million tons in 2012, and accounting for the 33.3% of the total world's strawberry production. Spain was the fourth larger producer, with the 7.1% of the total production; being surpassed by Mexico (8.8%), and Turkey (8.6%) (FAOSTAT, 2012).

Strawberries, mostly for the fresh market, are grown in other Spanish regions but production from the province of Huelva (Andalusia, South-Western Spain) dominates. More than 95% of this crop is located in this province. Climatic and soil characteristics make this area exceptional for strawberry production, allowing to supply the international markets early in the season (López-Aranda, 2008).

Strawberries in Huelva are usually grown on the same place year after year under unheated roll-up sided high plastic tunnels (Spanish tunnels); this semi-open cultivation system benefits growers by improving earliness (Hancock and Simpson, 1995; MacNaeidhe, 1999; López-Aranda, 2008). Planting system employs fresh short-day strawberry cultivars plants from high-elevation nurseries; usual plantation dates encompasses the second half of October, and harvest dates extend from the end of December to the first half of June. There is a registered surface of strawberry cultivation of 6600 ha in Huelva, with a production around 250 000 t (López-Aranda, 2008; Junta de Andalucía, 2011).

There are many arthropod pests that affect strawberries; but the two-spotted spider mite (TSSM), *Tetranychus urticae* Koch (Acari: Tetranychiidae), and the western flower thrips (WFT), *Frankliniella occidentalis* (Pergande) (Thysanoptera: Thripidae), are key pests of strawberries (Coll et al., 2007; Strand, 2008; Alvarado, 2009; Zalom et al., 2014). Strawberry plants are often simultaneously infested by both species, which can cause reductions both in yield and fruit quality when their population densities

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are high. TSSM feeding injuries produce a reduction of photosynthesis and transpiration, followed by reduction of total yield per plant, individual fruit size and number of fruits per plant. Damage to strawberry caused by WFT is characterized by brown and withered stigma and anthers, slight necrotic spots on the calyx of the flower, and reduction in flower receptacle size at high thrips density. WFT feeding causes net-like russeting in the surface of fruits, reducing shelf-life, and fruit appearance (Coll et al., 2007; Zalom et al., 2014). The small size, ethological traits, high reproductive potential, and ability to build up resistance to chemicals of both pests make difficult to manage them only with pesticides (Van Leeuwen et al., 2010; Zalom et al., 2014).

Pesticide residues have become a major worldwide issue resulting in legislative actions to limit and regulate their use. In Europe the Directive 2009/128/EC has regulated the availability and use of many active ingredients. The restricted numbers of chemicals approved for Integrated Pest Management (IPM) use in Andalusian strawberries (Junta de Andalucía, 2014), and the market pressures to reduce pesticide residues make non-chemical methods desirable for the control of strawberry pests (Strand, 2008; Alvarado, 2009). Today more than 60% of total strawberry production in Andalusia uses biological control as part of IPM strategies. Biological control techniques can reduce pesticide applications, making the crop environment safer to work in, and reducing pesticide residues (Bale et al., 2008). Augmentative techniques through releases of predatory mites and insects are usually used in horticulture, methods are well established, and some biocontrol agents and protocols are already in use for strawberries (Collier and Van Steenwyk, 2004; Rhodes and Liburd, 2006; López-Aranda, 2008; Pottorff and Panter, 2008). However, performance of biocontrol agents may differ between regions, cropping systems or crops (Collier and Van Steenwyk, 2004; Fitzgerald et al., 2007; Pottorff and Panter, 2008). Therefore, before recommending any strategy, it is necessary their evaluation in the field.

Spider mites and thrips are currently being controlled with the release of both specialist and generalist predators. Phytoseiidae is the most important family of acarine predators used in pest management programs (Gerson and Weintraub, 2007; Hoy, 2011). *Phytoseiulus persimilis* Athias-Henriot and *Neoseiulus californicus* (MacGregor) are among the species most used in horticultural and greenhouse crops, having been employed in strawberries for TSSM control (Fraulo and Liburd, 2007; Palevsky et al., 2008). *Amblyseius swirskii* (Athias-Henriot) is a Mediterranean phytoseiid species considered as a primary predator of thrips and whiteflies; it has been recommended and used to control TSSM as well as other pests in greenhouse and strawberry crops (Elsawi and Alazzazy, 2007; Gerson and Weintraub, 2007; Alvarado, 2009; Xu and Enkegaard, 2010; Sáenz-de Cabezón et al., 2010; Calvo et al., 2011; Kutuk et al., 2011). Species of the genus *Orius* (Heteroptera: Anthocoridae) are commonly found on cultivated and wild plants in the Mediterranean Europe, and have received much attention as biocontrol agents for thrips (Tommasini et al., 2004). Among them, *Orius laevigatus* (Fieber), the minute pirate bug, is a generalist predator commonly used to control thrips in greenhouses (Weintraub et al., 2011).

The main goals of this research were to determine if within a strategy of zero-residues for high-plastic tunnel grown strawberries the control of TSSM and WFT can be achieved by using the proposed species: *Phytoseiulus per similis*, *Neoseiulus californicus*, *Amblyseius swirskii*, and *Orius laevigatus*, and evaluate the relative effectiveness of the combined use of multiple predatory species in this environment.

MATERIAL AND METHODS

Study site and experimental design

Field trials were carried-out during two consecutive growing seasons using commercially available biocontrol agents intended to control TSSM and WFT at 'El Cebollar' Experimental Station (37º14'29" N, 06º48'03" W), 10 km NE of Huelva, Spain. Strawberry plants were cultivated according to local conventional grower practices, and recommendations established in the Spanish and Andalusian regulation for IPM strawberries (BOJA, 2008; Table 1). Short-day length strawberry 'Camarosa' (Bringhurst et al., 1994) was planted in the third week of October each year (winter plantation) in raised beds covered with black plastic mulch, and drip irrigation. Strawberry season extended until the last week of May in both years.

Table 1. Andalusian regulation for thrips and two-spotted spider (TSSM) mite in strawberry.

	Frankliniella occidentalis (Pergande)	*Tetranychus urticae* Koch[1]
Sampling	Examination 1 flower per plant, recording individual numbers of larvae and/or adults	Examination 2 leaves per plant, one young leaf and one older, recording adult females
Evaluation scale	0 = flower with < 3 mobile forms 1 = flower with ≥ 3 mobile forms	0 = No adult females 1 = ≥ 1 adult females
Economic injury levels (EIL)	70% of flowers with 3 or more mobile forms	15% leaves occupied until the end of February 25% leaves occupied in the rest of season if the leaves occupied by Phytoseiid are < 50% of leaves occupied by TSSM

[1]We have considered a leaf occupied when TSSM mobile forms were present.

Experimental design was a Latin square with four replicates by treatment in both years. Elementary plots had an area of 36 m^2 (12 m × 3 m), with 240 strawberry plants disposed in two rows on two raised beds. Two adjacent high tunnels (each 6.6 m wide × 60 m long) were used in the trials. Within each tunnel, plots were separated by an unplanted area of 3 m, and one raised bed of plants (buffer row). After transplanting, tunnels were covered with polyethylene plastic to keep the tunnel hot during the winter. Plants received no chemicals treatments during the trials. To minimize pesticide drifts from nearby plots in the farm, a vertical separation with polyethylene plastic supported by a metal frame was placed as a barrier on either side of the trial.

Phytoseiid species and *O. laevigatus* were supplied by Koppert España SL (Almería, Spain). *Phytoseiulus persimilis* was released from 100 mL bottles containing 2000 adults in sawdust. *Neoseiulus californicus* was supplied in 500 mL bottles containing 5000 mites in inert pellets. In 2009 *Amblyseius swirskii* was released as nymphs and adults from 500 mL bottles (50 000 mites); paper sachets with 250 mites plus storage mites mixed with bran were used in 2010. *Orius laevigatus* was supplied in 100 mL bottles containing a mix of 2000 adults and 5^{th} instar nymphs in buckwheat husks and vermiculite. All species were released as preventive treatments on the same day after their arrival to the farm following the guidelines established by the supplier. Species, combinations, doses and dates of releases are summarized up in Table 2.

Sampling and data analysis

Plots were sampled weekly, beginning approximately 1-mo after strawberries were planted and once new leaves were present. TSSM numbers were assessed by sampling two leaves (one young and another older) randomly selected taken from each of five randomly selected plants per plot (40 leaves per treatment), and recording all motiles present. WFT numbers were monitored by examining five randomly selected flowers per plot (20 per treatment), and recording all larvae and adults present. Each leaf or flower was checked in the field with the aid of 20X hand lens (Lenscope, Bausch & Lomb, Berlin, Germany).

Differences between TSSM and WFT numbers were separately analyzed in both years using Latin square ANOVA. If a significant ANOVA was detected, means were compared using one-tailed Dunnett's multiple comparisons test with a control (Quinn and Keough, 2006). *Phytoseiulus persimilis* alone was used as control treatment for TSSM analyses, and the treatment that included *O. laevigatus* was employed as control treatment for WFT analyses, because these are the species most commonly available and most commonly released in horticulture and biologically grown strawberries to control these pests (Heon and Park, 2006; Alvarado, 2009; Weintraub et al., 2011).

The economic injury levels (EILs) established in the Andalusian regulation for IPM strawberry production are based on percentage of occupation of leaves (TSSM), or flowers (WFT), and crop phase (Table 1; BOJA, 2008). Thus, within each strawberry season TSSM data were separated in three periods based on treatment dates and time during the season. Periods included: pre-treatment (3 wk prior to first treatment), early crop (post-treatment to end of February), and late crop (from 1 March to the last strawberry harvest of each year). WFT data were separated only into two periods based on treatment application dates of *O. laevigatus* and *A. swirskii*.

Performance and trend throughout the strawberry season were compared for each treatment and season using the percentages of occupation of leaves (TSSM) and flowers (WFT) by means of Kruskal-Wallis one-way nonparametric ANOVA followed by all-pairwise comparisons tests for mean separation (Z test, $\alpha = 0.05$).

Raw TSSM and WFT data were transformed before analyses using logarithmic transformation ($x' = \log(x+1)$) to meet the assumption of homogeneity of variance, and square root transformation ($x' = \sqrt{x+1}$) was applied to percentages. All statistical analyses were performed using Statistix 9.0 for Windows (Analytical Software, Tallahassee, Florida, USA).

Table 2. Biocontrol agents and combination treatments employed in the trial.

Biocontrol agent	2009			2010		
	Release date	Dose (ind. m^{-2})	Treatments	Release date	Dose (ind. m^{-2})	Treatments
Phytoseiulus persimilis (Pper)	17 December 2008 (week 51)	10	Pper Pper/Ncal Pper/Aswi Pper/Ncal/Olae	21 December 2009 (week 52)	10	Pper Pper/Ncal Pper/Ncal/Olae
Neoseiulus californicus (Ncal)	17 December 2008 (week 51)	15	Pper/Ncal Pper/Ncal/Olae	21 December 2009 (week 52)	15	Pper/Ncal Ncal/Aswi Pper/Ncal/Olae
	5 February 2009 (week 6)	15	Pper/Ncal Pper/Ncal/Olae	26 January 2010 (week 4) 16 February 2010 (week 7)	15 25	Pper/Ncal Pper/Ncal/Olae Ncal/Aswi
Orius laevigatus (Olae)	11 February 2009 (week 7)	3.5	Pper/Ncal/Olae	23 February 2010 (week 8)	3.5	Pper/Ncal/Olae
Amblyseius swirskii (Aswi)	11 February 2009 (week 7)	70	Pper/Aswi	23 February 2010 (week 8)	50	Ncal/Aswi

RESULTS

2008-2009 strawberry season

Natural population of TSSM was found in the trial. Mean TSSM density during the pre-treatment period was similar in all treatments ($F_{3,15}$ = 0.66; p = 0.605). A reduction in TSSM numbers was observed during the early phase of the crop in all treatments except the one in which *P. persimilis*, *N. californicus*, and *O. laevigatus* were released together; however, no significant differences were observed ($F_{3,15}$ = 1.97, p = 0.219). TSSM population suppression continued later in the crop season again without differences among treatments ($F_{3,15}$ = 0.92, p = 0.942; Figure 1A).

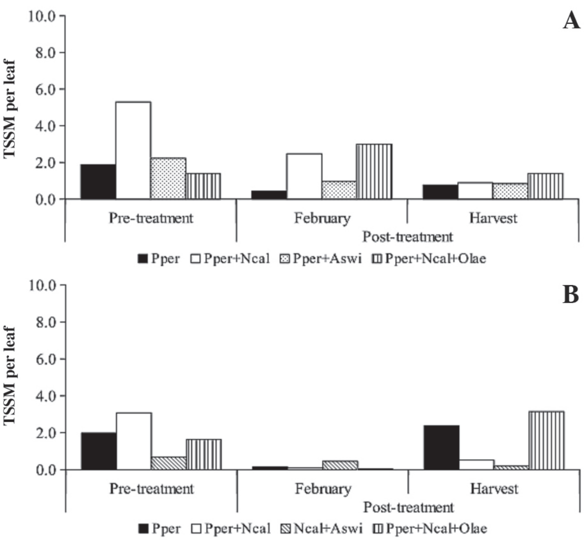
Percentages of leaves with TSSM decreased from pre-treatment to the end of the season in all treatments. No differences in the mean percentages of strawberry leaves with TSSM were found (Table 3). However, in the early crop phase, EIL was surpassed in four sampling dates for the treatment with *P. persimilis* + *N. californicus*; three dates in the combination *P. persimilis* + *N. californicus* + *O. laevigatus*; and two dates when *P. persimilis* was combined with *A. swirskii*. Late EIL was surpassed only in the treatment that combined *P. persimilis* + *A. swirskii*.

Mean WFT density was similar among treatments in 2009 before the releases ($F_{3,15}$ = 1.02; p = 0.447). WFT population increased more than 20 times after releases (0.21 ± 0.03 to 4.86 ± 0.18 thrips per flower). This increase was observed in all treatments. There were no significant differences in WFT numbers among treatments ($F_{3,15}$ = 1.92, p = 0.228; Figure 2A). The percentage of flowers with three or more thrips was less than 70% (EIL) in all treatments before releases. No difference in the total percentage of flowers with three or more thrips was observed at the end of the season (Table 4). The EIL was surpassed in the trial after the releases of *O. laevigatus* and *A. swirskii* from the first week of March to the second week of April. EIL was surpassed in eight sampling dates when *P. persimilis* was combined with *N. californicus*; seven dates in the treatment that combined *P. persimilis* + *A. swirskii*; six dates in the *P. persimilis* treatment; and four dates in the treatment that included *O. laevigatus*.

Table 3. Average percentages of leaves with two-spotted spider mite motiles in post-release phases during the two strawberry seasons considered in this study.

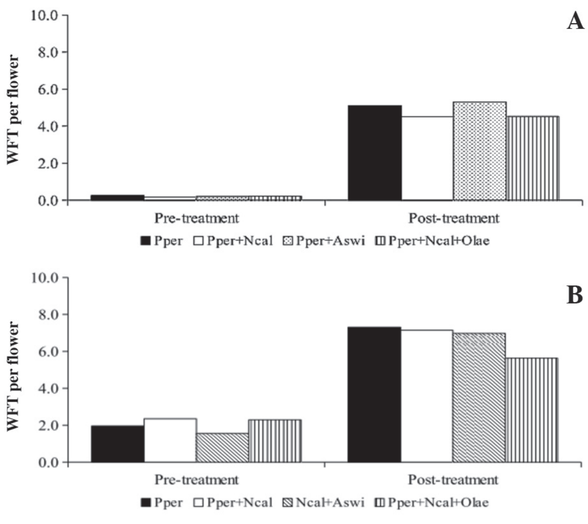
Treatments	2009 Early	2009 Late	2010 Early	2010 Late
Pper	7.78 ± 1.36a	10.21 ± 3.09a	1.43 ± 1.01a	16.04 ± 3.01a
Pper+Ncal	14.16 ± 3.61a	7.70 ± 2.77a	2.15 ± 0.92a	6.04 ± 2.55a
Pper+Aswi	12.22 ± 4.61a	8.33 ± 2.59a	-	-
Ncal+Aswi	-	-	7.50 ± 5.16a	6.25 ± 1.38a
Pper+Ncal+Olae	10.56 ± 3.32a	7.29 ± 2.29a	1.43 ± 0.00a	11.46 ± 4.77a
Kruskal-Wallis ANOVA	$F_{3,15}$ = 0.46 p = 0.718	$F_{3,15}$ = 0.19 p = 0.899	$F_{3,15}$ = 0.88 p = 0.481	$F_{3,15}$ = 2.41 p = 0.117

Treatments with the same letter are not significantly different from each other. Errors bars represent standard error of the mean.
Pper: *Phytoseiulus persimilis* alone; Pper+Ncal: *P. persimilis* + *Neoseiulus californicus* combination; Pper+Aswi: *P. persimilis* + *Amblyseius swirskii* combination; Ncal+Aswi: *N. californicus* + *A. swirskii* combination; Pper+Ncal+Olae: *P. persimilis* + *N. californicus* + *Orius laevigatus* combination.



Means with same letter are not significantly lower than the control treatment: Pper (one-tailed Dunnett's multiple comparison test, α = 0.05).
Pper: *Phytoseiulus persimilis* alone; Pper+Ncal: *P. persimilis* + *Neoseiulus californicus* combination; Pper+Aswi: *P. persimilis* + *Amblyseius swirskii* combination; Ncal+Aswi: *N. californicus* + *A. swirskii* combination; Pper+Ncal+Olae: *P. persimilis* + *N. californicus* + *Orius laevigatus* combination.

Figure 1. Two-spotted spider mite (TSSM) numbers during the different crop phases in the two strawberry seasons of study: 2009 (A) and 2010 (B). Data presented as means (± SE).



Pper: *Phytoseiulus persimilis* alone; Pper+Ncal: *P. persimilis* + *Neoseiulus californicus* combination; Pper+Aswi: *P. persimilis* + *Amblyseius swirskii* combination; Ncal+Aswi: *N. californicus* + *A. swirskii* combination; Pper+Ncal+Olae: *P. persimilis* + *N. californicus* + *Orius laevigatus* combination.
Means with same letter are not significantly lower than the control treatment: Pper+Ncal+Olae (one-tailed Dunnett's multiple comparison test, α = 0.05).

Figure 2. Western flower thrips (WFT) numbers during the different crop phases in the two strawberry seasons of study: 2009 (A) and 2010 (B). Data presented as means (± SE).

Table 4. Average percentages of flowers that surpassed economic injury level for western flower thrips (≥ 3 individuals per flower) during post-release phase of the two strawberry seasons.

Treatments	2009	2010
Pper	59.34 ± 1.39a	78.46 ± 2.59a
Pper+Ncal	61.33 ± 3.31a	71.92 ± 2.97ab
Pper+Aswi	65.67 ± 4.91a	-
Ncal+Aswi	-	76.15 ± 0.45ab
Pper+Ncal+Olae	56.67 ± 2.07a	66.15 ± 2.95b
Kruskal-Wallis ANOVA	$F_{3,15}$ = 0.88 p = 0.477	$F_{3,15}$ = 5.24 p = 0.015

Treatments with the same letter are not significantly different from each other. Errors bars represent standard error of the mean.
Pper: *Phytoseiulus persimilis* alone; Pper+Ncal: *P. persimilis* + *Neoseiulus californicus* combination; Pper+Aswi: *P. persimilis* + *Amblyseius swirskii* combination; Ncal+Aswi: *N. californicus* + *A. swirskii* combination; Pper+Ncal+Olae: *P. persimilis* + *N. californicus* + *Orius laevigatus* combination.

No Phytoseiids were observed in the trial before releases were done. *P. persimilis* mites were observed in the early and late crop seasons. The higher population of this mite was observed in the *P. persimilis* treatment (0.22 ± 0.10 mites per leaf), followed by the treatment *P. persimilis* + *A. swirskii* (0.12 ± 0.03). Population was lower in the *P. persimilis* + *N. californicus*, and similar to the treatment *P. persimilis* + *N. californicus* + *O. laevigatus*. No differences in *P. persimilis* numbers were observed among treatments ($F_{3,15}$ = 1.48, P = 0.248). *Phytoseiulus persimilis* numbers decreased in all treatments except in the *P. persimilis* + *A. swirskii* treatment from the early to the late crop phase. *Neoseiulus californicus* mites were observed in both periods of the crop season; it reached a population of 0.05 ± 0.02 mites per leaf in the early crop phase, and 0.02 ± 0.01 motiles per leaf in the late crop season. Few numbers of this mite were recorded in treatments in which no releases were done. *Neoseiulus californicus* was most abundant in the *P. persimilis* + *N. californicus* treatment (0.09 ± 0.04; $F_{3,15}$ = 4.88, p = 0.010). Despite *A. swirskii* being released only in combination with *P. persimilis*, the species was observed throughout the entire post-release period in others treatments. Very few mites were observed until the end of February, and although numbers increased late in the season, the abundance of this species was low (0.02 ± 0.01 mites per leaf). *Orius laevigatus* was observed in all treatments after release. Numbers were higher in the *P. persimilis* + *N. californicus* + *O. laevigatus* treatment (0.35 ± 0.03 bugs per flower). There were significant differences in *O. laevigatus* numbers among treatments at the end of the season ($F_{3,15}$ = 7.7, p = 0.017).

2009-2010 strawberry season

No difference in TSSM natural population density between both years was found ($F_{1,31}$ = 0.96, p = 0.335). TSSM numbers were also similar among treatments in the pre-treatment phase ($F_{3,15}$ = 0.48, p = 0.709). A reduction in TSSM numbers was observed in all treatments early in the season but without significant mean differences ($F_{3,15}$ = 1.00; p = 0.455). Late in the crop season TSSM numbers increased in the *P. persimilis* and *P. persimilis* + *N. californicus* + *O. laevigatus* treatments; however, once again no differences were found between treatments ($F_{3,15}$ = 1.64; p = 0.276; Figure 2A).

No difference in the mean percentage of leaves with TSSM motiles was observed. EIL was surpassed in the trial after the releases of biocontrol agents only in two sampling dates of the late crop phase in the treatment with *P. persimilis* (Table 3). WFT population in 2010 was 10X greater (2.04 ± 0.10 WFT per flower) than in 2009 (0.21 ± 0.03 WFT per flower) in the pre-treatment phase. WFT numbers were lower in plots when *N. californicus* + *A. swirskii* were released ($F_{3,15}$ = 16.17; p = 0.003). As in 2009, WFT population increased from March to the end of the strawberry season, this increase was lower in the treatment that included *O. laevigatus*; however, no significant differences were observed ($F_{3,15}$ = 1,92; p = 0.228; Figure 2B). Differences in mean percentage of flowers with three or more thrips were observed post-release. The percentage of flowers that surpassed EIL was higher when *P. persimilis* was released alone and lower when *O. laevigatus* was included in the combination (Table 4).

As in the 2008-2009 season, no Phytoseiids were observed in the trial before releases were done. *Phytoseiulus persimilis* reached an average of 0.07 ± 0.03 mites per leaf when released alone; *P. persimilis* numbers were lower in all the combination treatments, but there were not significant differences ($F_{3,15}$ = 0.30, p = 0.822). *Neoseiulus californicus* mites were observed in all treatments. This species reached an average of 0.06 ± 0.02 motiles per leaf at the end of the season. Higher values were observed in the *P. persimilis* + *N. californicus* + *O. laevigatus* treatment (0.10 ± 0.05 mites per leaf). The numbers of *N. californicus* did not differ among treatments ($F_{3,15}$ = 1.31, p = 0.295).

Very few *A. swirskii* mites were recorded in the 2009-2010 field season. The highest numbers of this species reached 0.04 ± 0.02 mites per leaf. *Amblyseius swirskii* numbers were higher in plots where it was initially released ($F_{3,15}$ = 4.70, P = 0.011). *Orius laevigatus* were also found in all treatments. The higher numbers of *O. laevigatus* were found in the *P. persimilis* + *N. californicus* treatment (0.31 ± 0.04 bugs per flower). There were no significant differences in *O. laevigatus* numbers among treatments at the end of the season ($F_{3,15}$ = 0.5, p = 0.693).

DISCUSSION

There are several studies that compare the effectiveness of combining multiple species releases to that of a single species in order to control single or multiple preys (Fitzgerald et al., 2007). Multiple natural enemy releases have been employed as a way to improve pest suppression also in strawberries (Rhodes et al., 2006; Cakmak et al., 2009; de Menten, 2011; Rahman et al., 2011). However, using multiple species may enhance or may reduce pest control, and there is no general agreement on whether the use of single or multiple biocontrol agents is economically profitable and beneficial for pest management (Gerson and Weintraub, 2007; Chow et al., 2010; Onzo et al., 2014). We found no significant differences when multiple predatory species were released to control TSSM numbers when compared with the release of *P. persimilis* alone. Schausberger and Walzer (2001) reported a similar situation with *P. persimilis* and *N. californicus* when released to control of *Tetranychus cinnabarinus* Boisduval on gerbera plants, and Cakmak et al. (2009) found that the control of this species on strawberries was not improved when these two species were released together. TSSM suppression varied among crop phases being proportionally greater in the early crop phase in both years. The levels of control of spider mite species attained by Phytoseiid predatory mites vary from species to species, and depend, among others factors, on predator/prey ratios (Hoy, 2011). Prey preference also plays an important role in the performance of polyphagous predators (Rasmy et al., 2004; Greco et al., 2005). Thus, when multiple species are present, there are some potential for trophic interactions (Walzer et al., 2001); cannibalism, inter- and intra-guild predation have been well documented for Phytoseiid mites and *Orius* species when the preferred prey level is low (Buitenhuis et al., 2009; Hoy, 2011). WFT and TSSM infesting cucumber were efficiently controlled with *Orius insidiosus* (Say) and *P. persimilis* (Fejt and Jarosik, 2000), van Baal et al. (2007) reported thrips control using *N. californicus* intended for TSSM control. However, when *O. insidiosus* was feed using either *F. occidentalis* or *T. urticae* separately higher suppression of the pest population was observed than when the two pests were simultaneously offered (Xu et al., 2006; Xu and Enkegaard, 2010).

Lower WFT numbers were found when *O. laevigatus* was released, but there were no significant differences in the reduction of WFT numbers among treatments. *Amblyseius swirskii* is a well known biocontrol agent of thrips' species in greenhouse crops (Wimmer et al., 2008; Arthurs et al., 2009); but in this study the release of this species did not resulted in significant reduction of WFT numbers. Variability in efficacy of *A. swirskii* on different crops has been related to trichome density (Buitenhuis et al., 2014). Previously, Elsawi and Alazzazy (2007) reported that *A. swirskii* successfully developed and reproduced on strawberry leaves feeding on TSSM, but that cultivar's characteristics affected behavior and other important life traits of this species such as longevity and fecundity. Poor adaptation to strawberry 'Camarosa', because of 'moderate' foliar pubescence (Voth et al., 1994) might be one feasible cause for explaining the low establishment, and lack of success of *A. swirskii* in our trials. Also, it is know that some *Orius* species will be prone to attack to any Phytoseiid present in strawberry flowers (Cloutier and Johnson, 1993); thus we can expect some degree of inter-specific competition with *O. laevigatus* when released together. Inter-guild competition through prey selection and predation may have played an important role in WFT control by *A. swirskii*.

The decrease in the numbers of WFT observed in the treatment with *O. laevigatus* was not sufficient to maintain WFT numbers below the present-day EIL for Andalusian strawberries. However, the early releases of *O. laevigatus* controlled thrips efficiently when the recommended EIL for winter planted strawberries in other countries (10 WFT per flower; Coll et al., 2007; Zalom et al., 2014) is applied. A low EIL for WFT such as the present-day on Andalusian strawberries (3 WFT per flower; BOJA, 2008) can result in unnecessary chemical treatments, which in turn can affect the implementation of other biological control strategies (Shakya et al., 2010). However, field data on the performance of natural enemies on controlling strawberry pests are still scarce in our country, and there is a lack of information on their comparative effectiveness.

Finally, high-plastic tunnels are not tight-enclosed controlled cropping systems such as high-tech greenhouses. The meteorological conditions of each season may play a significant role in the establishment of natural enemies and subsequent performance of biological control techniques. Dispersal or emigration from or into the crop is one of the factors limiting the effectiveness of biological control (Collier and Van Steenwyk, 2004). In our trials, Phytoseiids and *O. laevigatus* were recorded from treatments in which they were not initially released. Such situation, usual in open-field studies, has been mainly attributed to low prey density or absence of preferred prey (Rhodes et al., 2006). We suggest that some techniques to improve the establishment and maintenance of natural enemy populations in strawberry fields must be tested. Among these techniques the use of weed strips or banker plants seem to be appropriate in an augmentation/conservation strategy (Frank, 2010).

CONCLUSIONS

Our results support that control of two-spotted spider mite can be achieved in strawberries grown under high-plastic tunnels using the proposed species. Our data also support that early releases of *Orius laevigatus* seem to be a promising way to improve western flowers thrips control. However, there was not advantage in the release of multiple natural enemies, in the conditions of the present study. Therefore, further studies need to be carried out to determine the suitability of these and others species, combinatory techniques, and the release rates of each species that can effectively suppress pest population.

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